

AMENDMENTS TO THE SPECIFICATION

IN THE SPECIFICATION

On page 5, line 1, please replace the original paragraph with the following amended paragraph:

-- The present invention also provides a fructosylamine oxidase comprising the sequence:

GlyPhePhePheGluAlaAspGluAsnAsnGluLleLys (SEQ ID NO: 3).--

On page 5, line 4, please replace the original paragraph with the following amended paragraph:

-- The present invention provides a fructosylamine oxidase having any of the sequences of the following (e) to (h):

(e) PheHisTyrAspTyrValAlaProLeuAlaLysProAsnSerLysGluArg (SEQ ID NO: 4);

(f) AspAlaProLeuLeuHisAspLysGluTyrTyrGluGluLeuGlnLysAsnGlyLeuArgAsnTyrArg
TyrIleSerThr (SEQ ID NO: 5);

(g) ThrLysGlyAspLysGlyLeuAspProGluAspLys (SEQ ID NO: 6); and

(h) TrpValSerValGluAsnProThrProHisLysLeuGlu (SEQ ID NO: 7).--

On page 7, line 4, please replace the original paragraph with the following amended paragraph:

--Fig. 4 shows PCR primers used in the present invention (SEQ ID NOS: 8 – 15).--

On page 13, line 21, please replace the original paragraph with the following amended paragraph:

-- A fragment containing FAO structural gene may be amplified by a common method for the preparation of cDNA from mRNA by an RT-PCR. First, mRNA is subjected to a reverse transcription reaction using an oligo dT adapter primer according to the manual of an RNA-PCR kit (AMV Ver. 2.1, Takara). After cDNA is purified by ethanol precipitation, PCR is carried out using it as a template. Examples of the PCR primers include FAO-F1 which is designed from a conserved sequence of FAODs reported, and FAO-R2 which is designed from 13 amino acid residues obtained from amino acid sequence analysis of FAOD derived from the N1-1 strain.

FAO-F1 5'-GGXACXTGGGGXWSXWSXACXGCXYTXCA-3' (SEQ ID NO: 8)

FAO-R2 3'-TCYTCRTYXGGGYTCVAWRAARAAXCC-5' (SEQ ID NO: 9)

in which S = C + G, Y = C + T, R = A + G, X = A + C + G + T, W = A + T, V = A + C + G.—

On page 15, line 10, please replace the original paragraph with the following amended paragraph:

-- Then, PCR is carried out to analyze the nucleotide sequence of the C-terminal region. PCR is conducted using the purified cDNA as a template. Examples of the PCR primers include FAO-F3 designed from the sequence obtained from analysis of the nucleotide sequence of the insert and an adapter primer sequence.

FAO-F3 5'-ATTTCAAAGTGACGGATGAAGAAGCTAAAG-3' (SEQ ID NO: 10)

Adapter primer: 3'-CGCAGTTTTCCCAGTCACGAC-5' (SEQ ID NO: 11)--

On page 16, line 20, please replace the original paragraph with the following amended paragraph:

-- The genomic DNA derived from N1-1 strain may be used for amplification of FAOD structural gene fragment by inverse PCR. The genomic DNA is digested with a restriction enzyme and allowed for self-ligation according to the manual attached to DNA Ligation Kit Ver. 2. PCR is carried out using the resulting cyclic DNA as a template. Primers FAO-F5 and FAO-R6 designed from the partial sequence of the structural gene of FO are used as primers.

FAO-F5 5'-GTGCATACGAAGAATGCAAACGATTGGGAGTGG-3' (SEQ ID NO: 12)

FAO-R6 3'-CCATCCGTTATCTCCGTCGAGAACATATCCTC-5' (SEQ ID NO: 13)--

On page 17, line 19, please replace the original paragraph with the following amended paragraph:

-- The target gene may be obtained by analyzing the nucleotide sequence of the PCR amplified fragment. On the basis of the sequence information of FAO gene, a gene containing the full-length fragment may be prepared by PCR amplification from N1-1 genomic DNA. In order to amplify the sequence containing such gene regions, the following primers are designed.

FAO-NcoI: 5'-ATCACCATGGAGTCGATAATTATAGTTGG-3' (SEQ ID NO: 14)

FAO-XbaI: 3'-TTGATTCTAGACATGTATGTTGTAATCTTG-5' (SEQ ID NO: 15)--

On page 18, line 24, please replace the original paragraph with the following amended paragraph:

-- FAO derived from N1-1 strain may be recombinantly produced in *Escherichia coli*. Primers complementary to the N-terminal and C-terminal are designed from the structural gene sequence information of FAOD derived from N1-1 strain. In designing the primers, *Nco* I recognition sequence (FAO-*Nco* I) is added to a primer at the N-terminal side while *Xba* I recognition sequence (FAO-*Xba* I) is added to a primer at the C-terminal.

FAO-*Nco*I: 5'-ATCACCATGGAGTCGATAATTATAGTTGG-3' (SEQ ID NO: 14)

FAO-*Xba*I: 3'-TTGATTCTAGACATGTATGTTGTAATCTTG-5' (SEQ ID NO: 15)--

On page 26, line 9, please replace the original paragraph with the following amended paragraph:

-- The strain was incubated according to the publication "Screening and Characterization of Fructosyl-valine utilizing Marine Microorganisms" by K. Sode, et al., *Mar. Biotechnol.*, 3, 126-132 (2001). Purified enzyme of FAO derived from *Pichia* sp. N1-1 was obtained. A sample of the purified enzyme was freeze-dried and ultrapure water was added to 145 µg of the sample to the protein concentration of 2 mg/ml. The sample was subjected to SDS-PAGE (10% polyacrylamide gel) using a Lapidus-Slab electrophoretic device. The gel of the SDS-PAGE was cut out in a size of 5 × 5 cm and blotted to a PVDF membrane for 2 hours using Phast SystemTM. The PVDF membrane was stained with Coomassie Blue and the target band was cut out. The enzyme was digested with trypsin and separated by a reversed phase liquid chromatography. One of the fractions of the analyzed pattern was used for determination

of internal amino acid sequence on an amino acid sequencer (PPSQ-10 manufactured by Shimadzu). It was revealed that the enzyme contains a peptide sequence consisting of GlyPhePhePheGluAlaAspGluAsnAsnGluLeLys. (SEQ ID NO: 3)--

On page 28, line 5, please replace the original paragraph with the following amended paragraph:

-- A fragment containing FAO structural gene was amplified by a common method where cDNA is prepared by RT-PCR. First, mRNA was subjected to a reversed transcription reaction using an oligo dT adaptor primer according to the manual of RNA-PCR kit (AMV, Ver. 2.1, Takara). The cDNA was purified by precipitating with ethanol, and PCR was carried out using the cDNA. The PCR primers were FAO-F1 designed from a conserved sequence of already-reported FAODs and FAO-R2 designed from 13 amino acid residues obtained from amino acid sequence analysis of FAOD derived from N1-1 strain.

FAO-F1 5'-GGXACXTGGGGXWSXWSXACXGCXYTXCA-3' (SEQ ID NO: 8)

FAO-R2 3'-TCYTCRTYXGGYTCVAWRAARAAXCC-5' (SEQ ID NO: 9)

in which S = C + G, Y = C + T, R = A + G, X = A + C + G + T, W = A + T, V = A + C + G.--

On page 29, line 18, please replace the original paragraph with the following amended paragraph:

-- Then PCR was carried out to analyze the nucleotide sequence of the C-terminal region. PCR was conducted using the purified cDNA as a template. The PCR primers were

FAO-F3 designed from a sequence obtained by a nucleotide sequence analysis of the insert and a primer sequence of adapter primer.

FAO-F3: 5'-ATTTCAAAGTGACGGATGAAGAAGCTAAAG-3' (SEQ ID NO: 10)

Adapter primer: 3'-CGCAGTTTTCCCAGTCACGAC-5' (SEQ ID NO: 11)--

On page 31, line 1, please replace the original paragraph with the following amended paragraph:

-- The genomic DNA derived from the N1-1 strain was then used for amplification of FAOD structural gene fragment by inverse PCR. The genomic DNA was digested with a restriction enzyme and allowed for self-ligation according to the manual attached to DNA Ligation Kit Ver. 2. PCR was conducted using the resulting cyclic DNA as a template. The primers FAO-F5 and FAO-R6 designed from the partial sequence of the structural gene of FAO were used.

FAO-F5 5'-GTGCATACGAAGAATGCAAACGATTGGGAGTGG-3' (SEQ ID NO: 12)

FAO-R6 3'-CCATCCGTTATCTCCGTCGAGAACATATCCTC-5' (SEQ ID NO: 13)--

On page 31, line 22, please replace the original paragraph with the following amended paragraph:

-- TaKaRa LA *Taq*TM was used as a Taq polymerase. The PCR product was purified again and its nucleotide sequence was analyzed according to a common method. On the basis of FAO gene sequence information obtained above, a gene containing a full length

fragment was prepared by PCR amplification from the N1-1 genomic DNA. In order to amplify the sequence containing the gene region, the following primers were designed.

FAO-NcoI: 5'-ATCACCATGGAGTCGATAATTATAGTTGG-3' (SEQ ID NO: 14)

FAO-XbaI: 3'-TTGATTCTAGACATGTATGTTGTAATCTTG-5' (SEQ ID NO: 15)-

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On page 33, line 6, please replace the original paragraph with the following amended paragraph:

-- FAO derived from N1-1 strain was recombinantly produced using *Escherichia coli*. Primers complementary to the N-terminal and C-terminal were designed from the structural gene sequence information of FAO derived from N1-1 strain. In designing the primers, *Nco* I recognition sequence (FAO-Nco I) was added to the primer of the N-terminal side while *Xba* I recognition sequence (FAO-Xba I) was added to the primer of the C-terminal side.

FAO-NcoI: 5'-ATCACCATGGAGTCGATAATTATAGTTGG-3' (SEQ ID NO: 14)

FAO-XbaI: 3'-TTGATTCTAGACATGTATGTTGTAATCTTG-5' (SEQ ID NO: 15)--